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Advances in Brief

Molecular Analysis of the Cyclin-dependent Kinase Inhibitor Gene *p27/Kip1* in Human Malignancies[1]

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Abstract

Cyclin and cyclin-dependent kinase (CDK) complexes play important roles in controlling the cell cycle. The CDK inhibitors (CDKIs) inhibit the kinase activities of the complexes and block transitions of the cell cycle. Recently several CDKI genes have been cloned, and evidence suggests that at least a couple of these may be tumor suppressor genes. In this study, the partial structure of a CDKI gene, *p27/Kip1*, was determined. In addition, a large number of human cancers (432 cases) and cancer cell lines (20 lines) were analyzed for alterations of the *p27/Kip1* gene by Southern blot analysis and PCR/single-strand conformation polymorphism. The coding region of the *p27/Kip1* gene consists of at least two exons and an intron of about 600 bp. In 140 tumors of various tissues and 18 transformed cell lines, no deletions or rearrangements of the gene were detected by Southern blot analysis using a part of the coding sequence as a probe. One polymorphism and one silent mutation were detected by PCR/single-strand conformation polymoprhism. The polymorphism was a nucleotide substitution of guanine for thymine (GTC→GGC) at codon 109, resulting in an amino acid substitution of glycine for valine (Val→Gly). In summary, no abnormalities of the *p27/Kip1* gene were detected in human malignancies. Now, two groups of CDKIs are classified based on the structure of the proteins. One group includes the *p15, p16,* and *p18* CDKIs, which have ankyrin repeat motifs. The *p15* and *p16* CDKI genes are very frequently mutated in a variety of cancers. The *p27/Kip1* and *p21* CDKIs belong to the other group. We reported previously that abnormalities of the *p21* gene were very rare. The latter group of the CDKIs, including *p27/Kip1* and *p21*, are rarely mutated in human malignancies.

Introduction

During cellular proliferation, cyclins and CDKs[3] play important roles in the cell cycle. These proteins control transitions between G_1, S, G_2, and M phases of the cell cycle. In the transition from G_1 to S, cyclin Ds, CDK2, and CDK4 are key participants (1). Recently, several CDKIs, including p16/MTS1/INK4A/CDKN2 (2–4), p21/Waf1/Cip1/Sdi1/MDA6/CAP20 (5–8), p15/p14.5/MTS2/INK4B (4, 9–11, 27, 28), and p18 (11) have been cloned. The experimental overexpression of these CDKIs inhibits the kinase activities of the cyclin-CDK complexes and blocks the transition from G_1 to S, which inhibits cellular proliferation (12, 13). The CDKIs may be tumor suppressor proteins. The expression of p21 is induced by the tumor suppressor protein p53 (7). Abnormalities of p53 have been detected in nearly one-half of cancers (14, 15). Mutant p53 cannot regulate the *p21* gene and cell proliferation (7). Moreover, *p15* and *p16* genes also have abnormalities, especially deletions, in a variety of malignancies (3, 4). We postulated that *p27/Kip1*, one of the CDKIs recently cloned (16, 17), may be a tumor suppressor gene and may have mutations in several types of malignancies. In this study, we determined the partial structure of the *p27/Kip1* gene and analyzed the gene by Southern blot hybridization for structural alternations and by PCR-SSCP for point mutations in human malignancies.

Materials and Methods

Tissue Samples. Specimens were obtained from 36 non-small cell lung cancers, 20 ovarian cancers, 39 testicular cancers, 15 endometrial cancers, 18 prostate cancers, 31 gastric cancers, 61 cervical cancers (54 cases were HPV positive and 7 cases were negative), 73 sarcomas (48 osteosarcoma and 16 other sarcomas), 50 acute myelogenous leukemias and 82 acute lymphoblastic leukemias (Table 2). Matched normal DNA was available for the lung cancers and for some of the sarcomas. All tissues were collected at the time of surgery after informed consent by the patients. Twenty cell lines derived from a variety of malignancies were also analyzed (eight osteosarcoma cell lines, two testicular cancer cell lines, six HPV-positive cervical cancer cell lines, two HPV-negative cervical cancer cell lines, and two prostate cancer cell lines; Table 3).

Primers, PCR, and Sequencing. Sense and antisense primers were generated according to the cDNA and intronic sequences of the *p27/Kip1* gene. PCR was performed using Taq polymerase purchased from GIBCO-BRL (Gaitherburg, MD) under the condition of 1 min at 94°C for preheating, 30 cycles for 30 s denatured at 94°C, 1 min annealing at 60°C for regions Ia and Ib of exon I and at 55°C for exon II, 30-s extension at 72°C, and 10 min postextension at 72°C (18). The PCR products were cloned into pGEM-T vector (Promega, Madison, WI) and sequenced using specific primers by Sequenase version 7.0 kit (US Biochemicals, Cleveland, OH) or directly sequenced by the cycle sequence method (GIBCO-BRL).

Southern Blot Analysis. High molecular weight DNAs were extracted from cell lines and neoplastic and normal tissues by a standard proteinase K-phenol-chloroform extraction method (19). DNAs were digested by appropriate restriction enzymes and electrophoresed in 0.8% agarose gel and transferred to nylon membrane Hybond N+ (Amersham, Arlington Heights, IL) by alkali-transfer method according to the manufacturer's recommendation. Prehybridization, hybridization, and washing was performed as described previously (19). The probe for the *p27/Kip1* gene was generated by PCR using the SS2 primer listed in Table 1 and the A2 primer (5′ AGA ATC GTC GGT TGC AGG TCG CTT 3′), and the PCR product was gel purified. The probe was radiolabeled with [α-^{32}P]dCTP (NEN Research Products, Boston, MA) by the random priming method (19).

PCR-SSCP. The PCR amplification of each region was performed with the incorporation of [α-^{32}P]dCTP as described previously (20). The products were

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[3] The abbreviations used are: CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; PCR-SSCP, PCR/single-strand conformation polymorphism; HPV, human papilloma virus; MTS, multiple tumor suppressor; Waf, wild-type p53-activated fragment; Cip, Cdk-interacting protein; Sdi, senescent cell-derived inhibitor; MDA, melanoma differentiation associated; CAP, Cdk-associated protein.

Table 1 *Sequences of the primers*

S5 and A6 primers were used to clone the intronic sequence existing in the coding region. The others were used to amplify the coding region of the *p27/Kip1* gene.

Primer	Sequence
S5	5′-CGCAGGAATAAGGAAGCGACCT-3′
A6	5′-ACATTTTCTTCTGTTCTGTTGGCTC-3′
SS2	5′-ACCCGGGAGAAAGATGTCAAACGTG-3′
AA2	5′-TAGAACTCGGGCAAGCTGCCCTTCT-3′
SS3	5′-AGTACGAGTGGCAAGAGGTG-3′
AA3	5′-ATTCTATGGTTGGGGAAGGGT-3′
SS4	5′-GTTTTTTCTAATAAAGATTGTGTGTTC-3′
A7	5′-GTTTACGTTTGACGTCTTCTGAG-3′

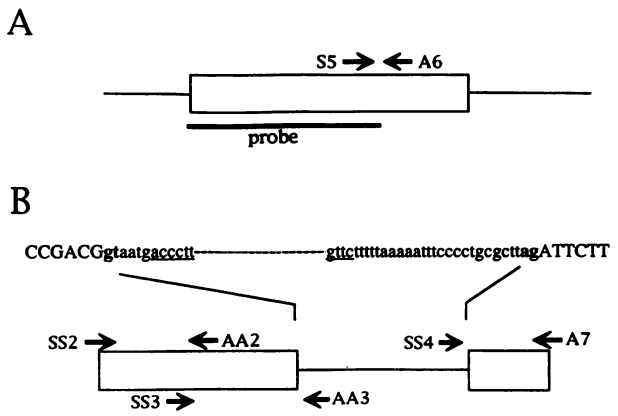

Fig. 1. *A*, cDNA structure of the *p27/Kip1* gene. The *box* indicates the coding sequence, and the *lines* indicate the 5′ and 3′ untranslated regions. *Arrows*, primers used to amplify the intronic sequence adjacent to the coding region. *Bold line*, a region used as a probe for Southern blot analysis. *B*, the partial genomic structure of the *p27/Kip1* gene. *Upper*, the exon-intron boundaries. *Capital letters* indicate the exon sequences, and *lowercase letters* show intronic sequences. The *underlined sequences* are part of the primer sequences, AA3 and SS4. The *bold lowercase letters* ("*gt*" in the left half and "*ag*" in the right half of the intron sequences) are compatible with the AG-GT spliced-site rule (21). *Lower*, Partial genomic structure of the *p27/Kip1* gene. *Boxes*, exons; a *line*, an intron. *Arrows*, the positions and direction of the primers. The 5′ and 3′ ends of the gene were not determined.

separated in 0.5X HydroLink MDE Gel (J. T. Baker, Inc., Phillipsburg, NJ) at room temperature. The gels were dryed and exposed to X-ray films at −80°C overnight.

Results

PCR-SSCP of the *p27/Kip1* Gene. To identify contigs of the cDNA sequence in the genome sequence, we used several pairs of primers from the cDNA sequence for PCR on normal genomic DNAs. Using primers S5 and A6, we obtained a product of approximately 600 bp in length (Fig. 1*A*), which was larger than expected from the cDNA sequence and thought to include the intronic sequence. Sequencing of this putative intron region showed that the proposed exon-intron boundary sequences are completely compatible with the "GT-AG " splice-site rule (Ref. 21; Fig. 1*B*). Other pairs of primers derived from the coding region yielded products as large as expected from the cDNA sequence (data not shown).

To look for point mutations by PCR-SSCP, the coding region of the *p27/Kip1* gene was divided into three regions [Ia, Ib (both in exon 1), and exon II], and three pairs of primers were constructed for PCR; four of these primers (SS2, AA2, SS3, and A7) were designed from the p27/Kip1 cDNA sequence, and two primers (AA3 and SS4) were constructed according to the intron sequences that we determined. The positions and directions of the primers are shown in Fig. 1*B*, and the primer sequences are listed in Table 1. The sizes of the amplified products were 276 bp for region Ia using SS2 and AA2 primers, 291 bp for region Ib using SS3 and AA3, and 177 bp for region II using SS4 and A7. For region II, no shifted bands were detectable in the human cancer samples and cell lines (Tables 2 and 3). For region Ia, a shifted SSCP band, which was not observed in the other cases, was detected in one gastric cancer (Fig. 2*A*; Table 2). The nucleotide sequence analysis revealed the nucleotide substitution of adenine for guanine at the codon 55 (GCG→ GCA) in the gastric cancer (Fig. 2*B*). This nucleotide substitution did not change the encoded amino acid

Table 2 *Analysis of the p27/Kip1 gene in a variety of human cancers*

Malignancy[a]	No. of samples	Abnormalities: Southern blot	Abnormalities: PCR-SSCP	Allele type[b]: A/A	A/B	B/B
Non-small cell lung cancer	36	0/17	0/36	23	11	2
Ovarian cancer	20	0/20	0/20	13	5	2
Testicular cancer	39	Not done	0/39	28	10	1
Endometrial cancer	15	0/15	0/15	11	4	0
Prostate cancer	18	Not done	0/18	16	2	0
Gastric cancer	31	Not done	1[c]/31 codon 55 (GCG → GCA)	30	1	0
Cervical cancer						
HPV(+)	51	0/28	0/51	38	10	3
HPV(−)	17	0/4	0/17	13	4	0
Osteosarcoma	48	0/40	0/48	29	16	3
Other sarcomas	25	0/16	0/25	17	7	1
AML	50	Not done	0/50	46	4	0
ALL	82	Not done	0/82	46	29	7
Total	432	0/140	1/432	310	103	19
				72%	24%	4%

[a] HPV(+), cervical cancer tissues which were positive for the human papilloma virus; HPV(−), cases negative for HPV. AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia.

[b] A/A, cases having homozygous alleles of the allele A (GTC at codon 109); B/B, cases having homozygous alleles of the allele B (GGC at codon 109); A/B, cases having heterozygous alleles.

[c] One case of gastric cancer had a point mutation at codon 55 changing GCG to GCA, but this nucleotide substitution does not change the amino acid residue (silent mutation).

Table 3 *Analysis of the p27/Kip1 gene in a variety of cell lines*

Cell lines[a]	Southern blot[b]	PCR-SSCP[b]	Allele type[c]
Osteosarcoma			
SAOS	−	−	B/B
U2OS	−	−	A/B
HOS	−	−	A/A
OSACL	−	Not done	Not done
MG63	−	Not done	Not done
G292	−	Not done	Not done
HT161	−	−	A/A
HS781	−	Not done	Not done
Testicular			
TERA-1	Not done	−	A/A
TERA-2	Not done	−	A/B
Cervical			
HPV(+)			
CaSki	−	−	A/A
C4I	−	−	B/B
HeLa	−	−	A/B
ME180	−	−	A/A
MS751	−	−	B/B
SiHa	−	−	A/A
HPV(−)			
C33A	−	−	A/A
HT3	−	−	A/B
Prostate cancer			
Du145	−	−	B/B
LnCap	−	−	A/A
Total			A/A A/B B/B
	0/18	0/16	8 4 4

[a] Twenty human cancer cell lines of various tissues were analyzed.

[b] −, no abnormalities found.

[c] Allele type A/A, A/B, and B/B refers to the respective allele types shown in the results and Table 2.

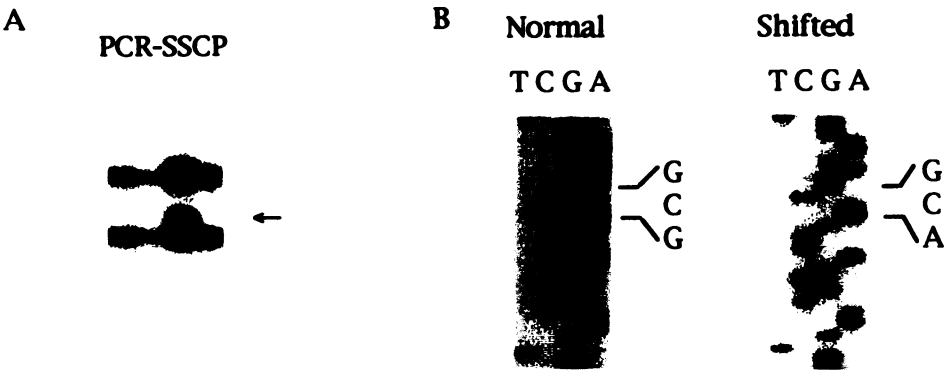

Fig. 2. Silent mutation of the *p27/Kip1* gene. *A*, the result of PCR-SSCP for exon 1. In each lane, two bands are normally detected. *Arrow*, a shifted band of one gastric cancer. *B*, the sequence of a normal sample and the sample with the shifted bands. At codon 55, a nucleotide substitution (GC*G*→GC*A*) is detected.

residues (silent mutation). Since matched normal tissues for this sample were unavailable, we could not identify whether the nucleotide substitution was a rare polymorphism or mutated during the process of malignant transformation.

For region Ib of exon 1, three patterns of bands occurred. Analysis using matched normal and neoplastic samples from the same patients showed that, in all cases, the patterns of the bands from the malignant and normal tissue of the same individual were identical (data not shown). This strongly suggested that the shifted bands were due to polymorphic nucleotide substitutions and not mutations. The polymorphism was a nucleotide substitution of guanine for thymine (GTC→GGC) at codon 109, resulting in an amino acid substitution of glycine (allele B) for valine (allele A; Val→Gly). The rate of homozygosity of allele A (GTC) was 72% (310 of 432), homozygosity of allele B (GGC) was 4% (19 of 432), and heterozygosity was 24% (103 of 432), as determined from 432 cases (Table 2).

Southern Blot Analysis for the *p27/Kip1* Gene. No deletions or rearrangements of the *p27/Kip1* gene were detected by Southern blot hybridization using a part of the coding region of *p27/Kip1* as a probe (Tables 2 and 3). The blots were also rehybridized with the vitamin D receptor gene as an internal control (data not shown).

Discussion

Southern blot analysis detected neither deletions nor rearrangements of the *p27/Kip1* gene; structural abnormalities of the coding region of the *p27/Kip1* gene are extremely rare in human cancers. We scanned the coding region of the *p27/Kip1* gene for point mutations using PCR-SSCP. We found only one silent mutation but could detect no mutations reflecting amino acid substitutions. The number and histological types of cancers that were analyzed in this study were large; therefore, we showed that abnormalities of the *p27/Kip1* gene are rare events in tumorigenesis in a diverse group of human cancers.

We have found one polymorphic nucleotide substitution, which resulted in an amino acid substitution at codon 109. The frequencies of polymorphisms are slightly different in each type of cancer (Table 2). These differences might be due to the geographic origin of the samples.

Recently, several CDKI genes have been cloned, and they are classified into two groups. One group consists of CDKIs having ankyrin repeat motifs, including p16/MTS1/INK4A/CDKN2 (2–4), p15/p14.5/MTS2/INK4B (4, 9–11), and p18 (11). These specifically inhibit the cyclin-CDK complexes involving CDK4 and CDK6, which are CDKs expressed exclusively at the mid-G_1 phase (2, 9, 11). Mutations and deletions of the *p16* and *p15* genes occur in a variety of human malignancies and transformed cell lines (3, 4, 22–26).

The other group consists of CDKIs, including p21/Waf1/Cip1/Sdi1/MDA-6/CAP20 (5–8, 27, 28) and p27/Kip1 (16, 17). These CDKIs have a 60-residue protein homology each other, which is important for their inhibitory activities (16, 17). Experimentally produced deletion mutants of *p27/Kip1* eliminating the homologous region do not have inhibitory activity. *p27/Kip1* inhibits the cyclin-CDK complexes involving a variety of CDKs including CDK2 and CDK4 (16, 17). CDK4 is expressed at the mid-G_1 phase preceeding expression of CDK2 (1). p21 is induced by the product of the *p53* tumor suppressor gene, which is known to be mutated in a variety of malignancies. We also could find no abnormalities of the *p21* gene in a variety of human cancers (20). CDKIs belonging to this group might be rarely mutated in human cancers.

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References

1. Sherr, C. J. Mammalian G1 cyclins. Cell, *73:* 1059–1065, 1993.
2. Serrano, M., Hannon, G. J., and Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature (Lond.), *366:* 704–707, 1993.
3. Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K.,and Carson, D. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature (Lond.), *368:* 753–756, 1994.
4. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. Science (Washington DC), *264:* 436–440, 1994.
5. Noda, A., Ning, Y., Venable, S. F., Pereira-Smith, O. M., and Smith, J. R. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Exp. Cell Res., *211:* 90–98, 1994.
6. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., and Beach, D. p21 is a universal inhibitor of cyclin kinases. Nature (Lond.), *366:* 701–704, 1993.
7. El-Deiry, W., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. Cell, *75:* 815–825, 1993.
8. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell, *75:* 805–816, 1993.
9. Hannon, G. J., and Beach, D. p15INK4B is a potential effector of TGF-β-induced cell cycle arrest. Nature (Lond.), *371:* 257–261, 1994.
10. Jen, J., Harper, W., Bigner, S. H., Bigner, D. D., Papadopoulos, N., Markowitz, S., Willson, J. K. V., Kinzler, K. W., and Vogelstein, B. Deletion of *p16* and *p15* genes in brain tumors. Cancer Res., *54:* 6353–6358, 1994.
11. Guan, K-L., Jenkins, C. W., Li, Y., Nichols, M. A., Wu, X., O'Keefe, C. L., Matera, A. G., and Xiong, Y. Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. Genes Dev., *8:* 2939–2952, 1994.
12. Hunter, T. Braking the cycle. Cell, *75:* 839–841, 1993.
13. Serrano, M., Gomez-Lahoz, E., DePinho, R. A., Beach, D., and Bar-Sagi, D. Inhibition of Ras-induced proliferation and cellular transformation by p16INK4. Science (Washington DC), *267:* 249–252, 1995.
14. Imamura, J., Miyoshi, I., and Koeffler, H. P. p53 in hematological malignancies. Blood, *84:* 2412–2421, 1994.
15. Prokocimer, M., and Rotter, V. Structure and function of p53 in normal cells and their aberrations in cancer cells: projection on the hematological cell lineages. Blood, *84:* 2391–2411, 1994.
16. Polyak, K., Lee, M-H., Erdjument-Bromage, H., Koff, A., Roberts, J. M., Tempst, P., and Massague, J. Cloning of p27 kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell, *78:* 59–66, 1994.

17. Toyoshima, H., and Hunter, T. p27, a novel inhibitor of G1 cyclin-cdk protein kinase activity, is related to p21. Cell, *78:* 67–74, 1994.
18. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science (Washington DC), *230:* 1350–1354, 1985.
19. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
20. Shiohara, M., El-Deiry, W. S., Wada, M., Nakamaki, T., Takeuchi, S., Yang, R., Chen, D-L., Vogelstein, B., and Koeffler, H. P. Absence of WAF1 mutations in a variety of human malignancies. Blood, *84:* 3781–3784, 1994.
21. Breathnach, R., and Chambon, P. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem., *50:* 349–383, 1981.
22. Mori, T., Miura, K., Aoki, T., Nishihara, T., Mori, S., and Nakamura, Y. Frequent somatic mutation of the CDKN2/CDK4I (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. Cancer Res., *54:* 3396–3397, 1994.
23. Hayashi, N., Sugimoto, Y., Tsuchiya, E., Ogawa, M., and Nakamura, Y. Somatic mutations of the MTS (multiple tumor suppressor) 1/CDK4I (cyclin-dependent kinase-4 inhibitor) gene in human primary non-small cell lung carcinomas. Biochem. Biophys. Res. Commun., *202:* 1426–1430, 1994.
24. Caldas, C., Hahn, S. A., da Costa, L. T., Redston, M. S., Schutte, M., Beymour, A. B., Weinstein, C. L., Hruban, R. H., Yeo, C. J., and Kern, S. E. Frequent somatic mutations and homozygous deletions of the *p16* (*MTS1*) gene in pancreatic adenocarcinoma. Nat. Genet., *8:* 27–32, 1994.
25. Xu, L., Sgroi, D., Sterner, C. J., Beauchamp, R. L., Pinney, D. M., Keel, S., Ueki, K., Rutter, J. L., Buckler, A. J., Louis, D. N., Gusella, J. F., and Ramesh, V. Mutational analysis of CDKN2 (MTS1/p16^{ink4}) in human breast carcinomas. Cancer Res., *54:* 5262–5264, 1994.
26. Ueki, K., Rubio, M-P., Ramesh, V., Correa, K. M., Rutter, J. L., von Deimling, A., Buckler, A. J., Gusella, J. F., and Louis, D. N. *MTS1/CDKN2* gene mutations are rare in primary human astrocytomas with allelic loss of chromosome 9p. Hum. Mol. Genet., *3:* 1841–1845, 1994.
27. Gu, Y., Turck, C. W., and Morgan, D. O. Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. Nature (Lond.), *366:* 707–710, 1993.
28. Jiang, H., and Fisher, P. B. Use of a sensitive and efficient subtraction hybridization protocol for the identification of genes differentially regulated during the induction of differentiation in human melanoma cells. Mol. Cell. Differ., *1:* 285–299, 1993.

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